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Efficacies of Antiherpesvirus Nucleosides against Two Strains of Herpes Simplex Virus Type 1 in Vero and Human Embryo Lung Fibroblast Cells

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Antiviral activities of five nucleoside analogs against the VR-3 and WT-34 strains of herpes simplex virus type 1 (HSV-1) were investigated in Vero and human embryo lung fibroblast (HEL) cells. In HEL cells, the compounds showed antiviral activities against both strains of HSV-1, but in Vero cells, the antiviral activities of the compounds were reduced in proportion to their antiviral indexes (the 50% inhibitory dose [ID_{50}] for cell growth divided by the 50% plaque reduction dose for virus). The ratio of the ID_{50} in Vero cells to the ID_{50} in HEL cells was larger in VR-3-infected cells than in WT-34-infected cells. The following results were obtained. (i) Thymidine kinase (TK; EC 2.7.1.21) activity in the VR-3- or WT-34-infected Vero cells was about half that in VR-3- or WT-34-infected HEL cells. Induction of viral TK was especially low in the VR-3-infected Vero cells. (ii) The ID_{50} of the plaque reduction assay in hypoxanthine, aminopterin, and thymidine medium revealed that the activity of cellular thymidylate synthetase (EC 2.1.1.45) was important in viral replication in VR-3-infected Vero cells. (iii) The VR-3-infected cells required larger thymidine and thymidine phosphate pools for viral replication than the WT-34-infected cells did, although uptake of 1-β-D-arabinofuranosyl-*E*-5-(2-bromovinyl) uracil into infected cells was equal for both strains. (iv) In the VR-3-infected Vero cells, the quantity of 1-β-D-arabinofuranosyl-*E*-5-(2-bromovinyl)uracil triphosphate was smaller than that in VR-3-infected HEL cells and WT-34-infected Vero and HEL cells.

In recent reports it has been shown that the efficacies of antiherpesvirus compounds vary with the cell lines used in antiviral activity assays (7, 13; H. Machida, T. Suzutani, and T. Sakuma, unpublished data). De Clercq (7) has reported that the 50% inhibitory doses (ID_{50}s) of compounds in human embryonic lung fibroblast (HEL) cells, which are generally used for antiviral tests, were relatively lower and that the ID_{50}s obtained in Vero cells were significantly higher than the ID_{50}s obtained in 10 other cell lines.

Reefschläger et al. (23) have reported the antiherpesvirus activity of 5-substituted 1-β-D-arabinofuranosyluracil analogs and other nucleosides in Vero and HEL cells (23). They observed that 1-β-D-arabinofuranosyl-*E*-5-(2-bromovinyl)uracil (BV-araU) and 1-β-D-arabinofuranosyl-5-vinyluracil were the most potent inhibitors of the 77 and V3 strains of herpes simplex virus type 1 (HSV-1) in HEL cells. However, these compounds were nearly 100 times or more less effective against the 77 and V3 strains in Vero cells compared with that in HEL cells. It is not known why these compounds were ineffective against HSV-1 in Vero cells, in spite of their efficacy in HEL cells, and why the ID_{50}s of these compounds in Vero cells differed significantly between the strains of HSV-1 compared with the lack of variation of the ID_{50}s in HEL cells.

The purpose of this study was to examine these problems.

MATERIALS AND METHODS

Compounds. BV-araU and 1-β-D-arabinofuranosylthymine (araT) were synthesized by Yamasa Shoyu Co., Ltd., Choshi, Japan. 5-iodo-2′-deoxyuridine (IDU) was purchased from Wako Fine Chemical Products, Ltd., Osaka, Japan. 9-β-D-Arabinofuranosyladenine (araA) was a gift from Mochida Pharmaceutical Co., Ltd., Tokyo, Japan, and 9-(2-hydroxyethoxymethyl)guanine (ACV) was supplied by Japan Wellcome Co., Ltd., Osaka, Japan. [*methyl*-^{3}H] thymidine (83 Ci/mmol) and deoxy[5-^{3}H]UMP (10.6 Ci/mmol) were purchased from the Radiochemical Center, Amersham, England. [*bromovinyl*-^{14}C]BV-araU (4.12 mCi/mmol) was synthesized by Daiichi Pure Chemicals, Ltd., Tokyo, Japan, with a radiochemical purity of 99.3% by thin-layer chromatography and 99.9% by high-performance liquid chromatography (HPLC).

Cells and viruses. HEL cells, strain HAIN-55 (19), were kindly supplied by H. Okumura, National Institute of Health of Japan. Vero cells were obtained from Flow Laboratories, Inc., McLean, Va. These cells were cultivated in Eagle minimum essential medium (MEM) supplemented with 10% fetal bovine serum. The VR-3 strain of HSV-1 described previously (8) was obtained from the American Type Culture Collection, Rockville, Md. The WT-34 strain was a freshly isolated virus from a patient with herpes keratitis and was kindly supplied by T. Kurimura, Tottori University School of Medicine, Yonago, Japan. The VR-3 strain was passaged many times in cultured cells, eggs, and mouse brain; and the WT-34 strain was passaged less than 5 times in HEL cells before it was supplied to our laboratory. Therefore, these strains are referred to as the laboratory strain and the clinically isolated strain, respectively. Each strain was cloned 3 times and grown in HEL cells for less than three passages in our laboratory. All viruses were stored in small portions at −80°C.

Assay of inhibitory effect of compounds on cell growth. The

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cells were seeded in 24-well plastic plates at 2×10^4 per well. After 1 day, the cells were refed with MEM–10% fetal bovine serum containing an appropriate amount of the test compound. After incubation for 2 days, cells were dispersed by treatment with trypsin, and the viable cell numbers were counted. The 50% effective dose for cell growth (ED_{50}) was determined graphically.

Plaque reduction assays. The effect of each antiviral compound on the replication of HSV-1 was evaluated by the plaque reduction assay described previously (16).

To investigate the effect of dTMP on the antiviral activities of the compounds, the ID_{50} was measured in cells in which thymidylate synthetase (TS; EC 2.1.1.45) was inhibited by aminopterin as follows; the infected cells were overlayed with medium containing hypoxanthine, aminopterin, and thymidine (HAT medium). In preliminary experiments, the concentrations in HAT medium were 100 μM hypoxanthine, 0.4 μM aminopterin, and 1.6 μM thymidine.

Assay of thymidine kinase activity. The VR-3-, WT-34- and mock-infected cell extracts were evaluated for thymidine kinase (TK; EC 2.7.1.21) activity as follows. Cells were infected with virus at a multiplicity of infection of 2, and after 1 h of incubation at 37°C, they were washed with MEM and replenished with MEM–2% fetal bovine serum and cultivated for the desired periods. The cells were washed, suspended in 1 ml of TMT buffer (50 mM Tris hydrochloride [pH 7.5], 5 mM 2-mercaptoethanol, 5 μM thymidine), and disrupted in a sonicator (Kontes, N.J.) for 30 s at full power. The sonicated cells were centrifuged at $10,000 \times g$ for 30 min at 4°C, and the supernatant was used as the crude enzyme material. The TK assay was carried out as described previously (14, 25) by using [*methyl*-^{3}H]thymidine as the substrate.

Studies of the inhibition of TK activity by BV-araU were performed by using the same conditions as described above for the TK assay, except that various concentrations of BV-araU were added to the reaction mixture.

Assay of TS activity. HSV-1-infected and mock-infected cells were washed once with MEM and suspended in 0.5 ml of 50 mM Tris hydrochloride (pH 7.5) containing 10 mM dithiothreitol and 0.1% Triton X-100. The cells were disrupted in a sonicator (Kontes), and the supernatants that were obtained as described above were used for the crude TS enzyme preparations. The TS activity was assayed by previously described methods (3, 24).

Uptake and turnover of thymidine and BV-araU in cells. Confluent HEL or Vero cell monolayers in petri dishes (diameter, 52 mm) were either mock infected or infected with HSV at a multiplicity of infection of 2. After 1 h of incubation at 37°C, 2 ml of MEM–2% fetal bovine serum containing 2 μCi of [*methyl*-^{3}H]thymidine or [*bromovinyl*-^{14}C]BV-araU was added. The fetal bovine serum used in this experiment was dialyzed against Earle solution. At the indicated times the cells were washed twice with MEM and harvested by trypsinization. The cell suspensions were centrifuged and washed once with MEM. The cell pellet was suspended in 0.4 ml of ice-cold distilled water and disrupted by sonication. To all samples, 2 ml of cold 10% (wt/vol) trichloroacetic acid was added and kept for 15 min in an ice bath. The trichloroacetic acid-soluble fractions were separated from the acid-insoluble DNA fraction by centrifugation at 3,000 rpm for 10 min. The pellets were suspended in 5% trichloroacetic acid, and after 15 min of incubation at 90°C, the soluble DNA fraction was separated from the protein residue by centrifugation. Then, the acid-soluble fractions or the DNA fractions were mixed with Univer Gel II [2,5-diphenyloxazole, 1,4-bis(5-phenyloxazolyl)benzene, and nonionic surfactant in xylene; Nakarai Chemicals, Ltd., Kyoto, Japan], and the radioactivity was measured in a liquid scintillation counter.

Thin-layer chromatography. The various nucleosides from the acid-soluble fractions described above were separated by polyethyleneimine-cellulose thin-layer chromatography (Macherey-Nagel Co., Ltd., Düren, Federal Republic of Germany). Before use, thin-layer plates were washed thoroughly as described previously (22) and stored at 4°C in the dark until use. Five microliters of sample, which was neutralized with 1 M KOH, was spotted onto the plates and developed in a solvent composed of 1 M LiCl and 1 M formic acid. After the plates were dried, they were cut into pieces (2 by 2 cm) and analyzed for radioactivity.

HPLC. Degradation of BV-araU to (*E*)-5-(2-bromovinyl)uracil was analyzed by HPLC. Monolayers of HEL or Vero cells were mock infected or infected with HSV at a multiplicity of infection of 2. After adsorption for 1 h, MEM–2% fetal bovine serum containing 25 μg of BV-araU per ml was added. The concentrations of BV-araU and *E*-5-bromovinyluracil in the culture medium at 96 h of incubation were determined by HPLC on a reverse-phase Chemopak 300-10C18 column in a Shimadzu LC-5A chromatographic system. The detectable amount of nucleoside in this assay was about 0.06 μg/ml for each compound under the conditions of analysis described previously (26). The retention time of *E*-5-bromovinyluracil and BV-araU in this system were 7.94 and 9.64 min, respectively.

RESULTS

Anticellular and antiviral activities of compounds in Vero and HEL cells. The effects of the compounds on cell growth were examined. Marked differences were not observed between the ED_{50} for Vero cells and the ED_{50} for HEL cells (Table 1). The ID_{50}s for the VR-3 and WT-34 strains of HSV-1 are shown in Table 2. In HEL cells, the VR-3 and WT-34 strains were susceptible to all compounds tested. The ID_{50} for the VR-3 strain was almost equal to that for the WT-34 strain, and the difference was less than fourfold for the two strains. However, in Vero cells the ID_{50}s for the two strains of HSV-1 were higher than those in HEL cells, and the largest ratio of ID_{50} (Vero)/ID_{50}(HEL) was for the VR-3 strain. In particular, the ratio for BV-araU was more than 14,000, and those for araT and ACV were 65 and 13, respectively. In contrast with the VR-3 strain, all tested compounds had a similar effect against the WT-34 strain in Vero cells, and the ID_{50}(Vero)/ID_{50}(HEL) ratios were all less than 10. The ratio of ID_{50}(Vero)/ID_{50}(HEL) correlated with the antiviral index ratio, in agreement with results of an earlier report (16).

As shown above, the antiviral activities of the compounds, especially BV-araU, were markedly different, depending on

TABLE 1. Susceptibilities of Vero and HEL cells to the inhibitory effects of compounds

Compound	ED_{50} (μM) for:	
	Vero cells	HEL cells
BV-araU	730	1,000
araT	360	240
IDU	33	15
ACV	190	530
araA	23	23

TABLE 2. Susceptibilities of the VR-3 and WT-34 strains of HSV-1 to the inhibitory effects of compounds in Vero and HEL cells

Virus strain	Compound	ID_{50} (μM) for:			Antiviral index for[a]:		
		Vero cells	HEL cells	Vero/HEL[b]	Vero cells	HEL cells	HEL/Vero[b]
VR-3	BV-araU	>1,100[c]	0.080	>14,000	<0.66	12,800	>19,400
	araT	43	0.66	65	8.3	808	97
	IDU	18	3.7	4.9	1.8	4.1	2.3
	ACV	4.9	0.39	13	39	41	1.1
	araA	67	18	3.7	0.34	1.3	3.8
WT-34	BV-araU	0.17	0.024	7.1	4,300	42,600	9.9
	araT	1.6	0.33	4.8	222	736	3.3
	IDU	5.6	3.5	1.6	5.9	4.3	0.73
	ACV	0.89	0.40	2.2	216	1,330	6.2
	araA	10	25	0.4	2.3	0.92	0.4

[a] Ratio of ED_{50} for cells (data from Table 1) to ID_{50} for viruses in the respective cells.
[b] Ratio of ID_{50} or antiviral index in Vero to HEL cells.
[c] This was the maximum concentration of BV-araU which could be added to the medium.

which virus strain and cell line was used, although the cell toxicity of these compounds was almost the same for both cell lines. Therefore, the growth of both strains of HSV-1 in Vero and HEL cells was investigated. At 48 h postinfection, the titers of the VR-3 strain were 2.8×10^7 and 2.2×10^7 PFU/10^6 cells and the titers of the WT-34 strain were 1.2×10^7 and 3.3×10^7 PFU/10^6 cells in Vero and HEL cell cultures, respectively. Each virus strain replicated equally well in both cell lines, even though the sensitivity of the VR-3 strain to the compounds differed greatly between the cell lines.

Degradation of compounds in culture medium of HSV-1-infected cells. In order to determine whether the extent of degradation of the compounds differed between the cell lines infected with the different virus strains, the overlay medium containing the compound was replenished daily. No difference in the ID_{50}s was observed whether the cultures were refed daily or not (data not shown). Then, the consumption of BV-araU in the medium was analyzed by HPLC. Mock- or HSV-1-infected cells were cultured in medium containing 25 μg of BV-araU per ml, and after 96 h of incubation, the amount of BV-araU in the medium was determined. The concentration of BV-araU was almost equal to that of the control in all cases, suggesting that there was no detectable consumption of BV-araU in the medium (data not shown). These results indicate that the degradation of BV-araU in the medium, even if it occurred, was not an important factor causing the difference of susceptibilities to compounds between cell lines infected with different virus strains.

TK activities in HSV-1-infected cells. It is well known that, except for araA, the compounds used in this study were phosphorylated by viral TK, cellular TK, or both. Also, the compounds had an ID_{50}(Vero)/ID_{50}(HEL) ratio which correlated with the antiviral index (Table 2) (16). Hence, the TK activities in HSV-1-infected cells were assayed in order to determine whether TK provides an important contribution to the ID_{50}(Vero)/ID_{50}(HEL) ratio. The TK activities in the VR-3-infected HEL cells were approximately equal to those in the WT-34-infected HEL cells (Fig. 1a), and these activities were about 14 times higher than those in mock-infected HEL cells. In the case of Vero cells, TK activities in virus-infected cells were about a half of those in HEL cells, and the TK activities in VR-3-infected cells were about 25% lower than those in WT-34-infected cells (Fig. 1b).

To estimate the amount of viral TK in the crude cell extract, it was assayed with BV-araU added to the reaction mixture (Fig. 2). The TK activities of mock-infected Vero and HEL cells were not inhibited by 400 μM BV-araU (the reaction mixture contained 1.7 μM thymidine). The TK activities of both VR-3- and WT-34-infected Vero and HEL cells, however, were markedly inhibited by BV-araU. Cellular TK activities in VR-3-infected HEL cells and WT-34-infected Vero and HEL cells were less than 16% of the total TK activity, while the cellular TK activities in VR-3-infected Vero cells were less than 34% of the total TK activity (Fig. 2). From these results and the results shown in Fig. 1b, the viral TK activities in VR-3-infected Vero cells were estimated at about 60% of those in WT-34-infected Vero cells.

Antiviral activities of compounds in Vero and HEL cells with TS inhibited by aminopterin. The main biosynthetic pathway of dTMP in normal cells is by the conversion of dUMP by TS, although the conversion of thymidine to dTMP by TK is also active in HSV-1-infected cells. The results presented above indicate that viral TK activities in VR-3-infected Vero cells were markedly lower than those in WT-34-infected Vero cells, and that the antiviral activities of compounds in VR-3-infected Vero cells were much lower than those in WT-34-infected Vero cells. These results suggest an important role for cellular TS, but not TK, in the

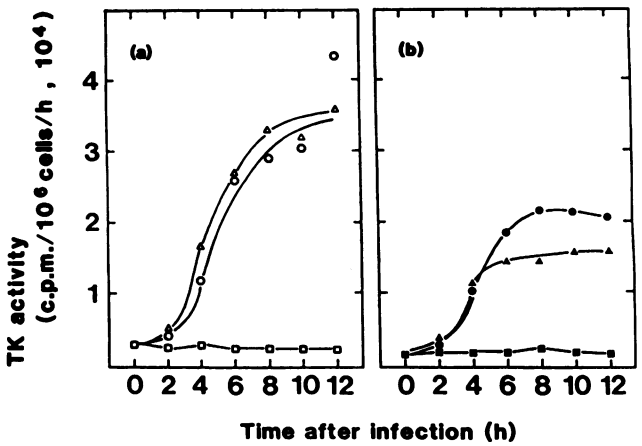

FIG. 1. TK activities in VR-3 or WT-34-infected cells. (a) Symbols: □, mock-infected HEL cells; △, VR-3-infected HEL cells; ○, WT-34-infected HEL cells; (b) Symbols: ■, mock-infected Vero cells; ▲, VR-3-infected Vero cells; ●, WT-34-infected Vero cells.

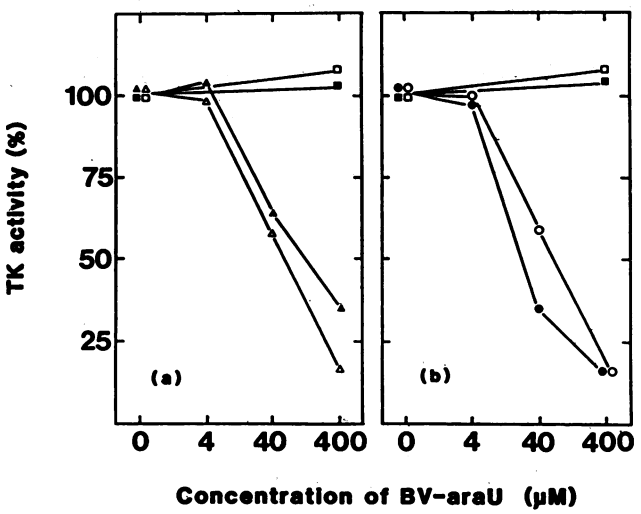

FIG. 2. Inhibition of TK activity by BV-araU. TK activity was assayed with BV-araU added to the reaction mixture containing 1.7 μM thymidine. Symbols: □, mock-infected HEL cells; ■, mock-infected Vero cells. (a) Inhibition of TK activity in the VR-3-infected cells. Symbols: △, VR-3-infected HEL cells; ▲, VR-3-infected Vero cells. (b) Inhibition of TK activity in the WT-34-infected cells. Symbols: ○, WT-34-infected HEL cells; ●, WT-34-infected Vero cells.

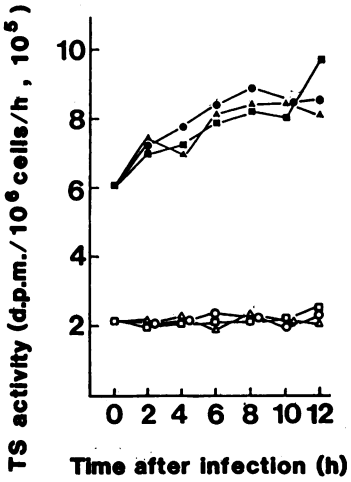

FIG. 3. TS activities of HSV- and mock-infected cells. Symbols: □, mock-infected HEL cells; ■, mock-infected Vero cells; △, VR-3-infected HEL cells; ▲, VR-3-infected Vero cells; ○, WT-34-infected HEL cells; ●, WT-34-infected Vero cells.

biosynthesis of dTMP in VR-3-infected Vero cells. Therefore, in order to investigate the effects of cellular TS on the antiviral activities of compounds, the ID_{50}s were measured with HAT in the medium. The results presented in Table 3 indicate that BV-araU and araT were more effective against the VR-3 strain in Vero cells in HAT medium than in control medium and that the ratio of ID_{50}(Vero)/ID_{50}(HEL) of BV-araU and araT to the VR-3 strain decreased more than 40 and 59 times, respectively, in the presence of HAT. In WT-34-infected-Vero cells, araT was remarkably effective in HAT medium, and the ID_{50}(Vero)/ID_{50}(HEL) ratio became very low. No major change was observed in the other cases.

When the TS activities of VR-3-, WT-34-, and mock-infected cell extracts were assayed (Fig. 3), no differences were observed. However, the TS activities of the VR-3-, WT-34-, and mock-infected Vero cells were 3 times higher than those of HEL cells.

Uptake and turnover of thymidine and BV-araU in HSV-1-infected cells. The uptake of [*methyl*-^{3}H]thymidine and [*bromovinyl*-^{14}C]BV-araU into the acid-soluble (nucleoside and nucleotide pools) and acid-insoluble (DNA) fractions of VR-3-, WT-34-, or mock-infected cells was measured. The uptake of nucleosides into the acid-soluble fraction was enhanced by virus infection (Table 4). In the case of BV-araU, the difference in the amount of uptake for VR-3-infected Vero cells was twice that for WT-34-infected Vero cells, even though the quantity of thymidine in the acid-soluble fraction of VR-3-infected cells was 7 to 12 times higher than that in WT-34-infected cells. Nevertheless, no difference in thymidine incorporation in the acid-insoluble fraction was observed between the two virus strains.

The turnovers of thymidine and BV-araU in VR-3- and WT-34-infected cells were analyzed by thin-layer chromatography (Table 5). In VR-3-infected Vero and HEL cells, the quantity of thymidine triphosphate was 7 to 10 times higher than that in these cells infected with WT-34. Thymidine was phosphorylated to thymidine triphosphate at a rate of 6 to 9% of incorporated thymidine in both VR-3- or WT-34-infected cells. In VR-3-infected Vero cells, however,

TABLE 3. Effect of HAT medium on susceptibilities of the VR-3 and the WT-34 strains in Vero and HEL cells to the inhibitory effects of compounds

Virus strain	Compound	ID_{50} (μM) in HAT medium for[a]:		Vero/HEL[b]		
		Vero cells	HEL cells	HAT(−)[c]	HAT(+)	HAT(−)/HAT(+)
VR-3	BV-araU	290	0.83	>14,000	350	>40
	araT	3.0	2.7	65	1.1	59
	IDU	31	24	4.9	1.3	3.8
	ACV	8.0	1.2	13	6.7	1.9
	araA	110	22	3.7	5.0	0.74
WT-34	BV-araU	0.37	0.066	7.1	5.6	1.3
	araT	0.0099	2.4	4.8	0.0041	1,200
	IDU	10	18	1.6	0.56	2.9
	ACV	2.1	0.80	2.2	2.6	0.85
	araA	37	32	0.4	1.2	0.33

[a] The concentrations of hypoxanthine, aminopterin, and thymidine were 100, 0.4, and 1.6 μM, respectively.
[b] Ratio of ID_{50} in Vero cells to that in HEL cells in the absence [HAT(−)] and the presence [HAT(+)] of HAT.
[c] Ratios are from Table 1.

TABLE 4. Uptakes of [*methyl*-^{3}H]thymidine and [*bromovinyl*-^{14}C]BV-araU in VR-3-, WT-34-, and mock-infected cells

Compound	Cell	Virus strain	Amt (pmol/10^6 cells) of uptake in[a]:	
			Acid-soluble fraction	Acid-insoluble fraction
Thymidine	Vero	Mock	69.6	895
		VR-3	3,530	1,020
		WT-34	285	846
	HEL	Mock	28.1	20.7
		VR-3	6,440	429
		WT-34	879	390
BV-araU	Vero	Mock	9.7	6.5
		VR-3	4,010	24
		WT-34	2,270	18.1
	HEL	Mock	12.6	4.0
		VR-3	9,150	28.6
		WT-34	8,020	30.9

[a] Amount of uptake of thymidine or BV-araU in acid-soluble or acid-insoluble fractions of VR-3-, WT-34-, or mock-infected cells.

only 1% of the BV-araU that was incorporated into the acid-soluble fraction was phosphoryrated to triphosphate; this value was 6% in WT-34-infected Vero cells.

DISCUSSION

In this study we examined the reasons why the antiherpesvirus activities of compounds varied from one cell line to another, depending on the strain of HSV-1 with which they were infected. Results of preliminary unpublished experiments showed that three laboratory strains of HSV-1, including VR-3, had a high ID_{50}(Vero)/ID_{50}(HEL) ratio for many compounds, but two clinical isolates of HSV-1, including WT-34, and four strains of varicella-zoster virus only had a ratio of about 1. Results of the present study showed that four compounds (BV-araU, araT, IDU, ACV), which are phosphorylated by viral TK, cellular TK, or both, are less effective in Vero cells than in HEL cells and are also less effective in VR-3-infected Vero cells than in WT-34-infected Vero cells (Table 2). Since this phenomenon was related to the antiviral indexes (ratio of ED_{50} for cells to ID_{50} for virus replication in those cells) of these compounds (Table 2) (16), we examined the steps by which these compounds were converted to the diphosphate form.

The first step in the conversion is catalyzed by TK. Total TK activities were about 2 times higher in VR-3- or WT-34-infected HEL cells than those in VR-3- or WT-34-infected Vero cells (Fig. 1). Similar results have been reported by Harmenberg et al. (12). Our study of the inhibition of TK activity by BV-araU showed that BV-araU was phosphorylated only by viral TK, which was elevated in VR-3- and WT-34-infected cells (Fig. 1). The results that showed that the ratio of ID_{50} (Vero)/ID_{50}(HEL) for araA was the lowest of the tested compounds in both strains (Table 2) support the suggestion that TK activity was involved in the ID_{50}(Vero)/ID_{50}(HEL) ratio of other compounds, since araA is phosphorylated only by cellular enzymes (20).

The second step in phosphorylation is catalyzed by viral TK, cellular thymidylate kinase, or both, except for ACV and araA (4, 9, 11, 18, 20). In this step, TS activity is important, because dTMP, which is synthesized from dUMP by TS, inhibits the phosphorylation of the compounds to the diphosphate form by direct competition (Table 5) (2). The results of the plaque reduction experiment with HAT medium indicated that BV-araU and araT were effective in VR-3-infected Vero cells in the presence of HAT (Table 3). Also, the TS activities of Vero cells were over 3 times those of HEL cells (Fig. 3). Although thymidylate kinase activity was not assayed in this study, the results of the measurement of TK activity (Fig. 1) suggest that the reduced phosphorylation of BV-araU to BV-araU diphosphate and triphosphate in VR-3-infected Vero cells (Table 5) may also be caused by the difference of viral-specific TK activities between VR-3- and WT-34-infected Vero and HEL cells. In summary, in the case of VR-3- and WT-34-infected Vero cells, there was a large pool of dTMP and less enzyme activity which phosphorylated the antiherpesvirus compound monophosphate to diphosphate form; hence, antiherpesvirus compounds are less effective against VR-3 and WT-34 in Vero cells than in HEL cells.

Furthermore, the antiherpesvirus activities of compounds was very much lower for VR-3 than for WT-34 in Vero cells (Table 2). This phenomenon might be due to the same mechanism as that which has been proposed to explain the low antiherpesvirus activities in Vero cells. Although TK

TABLE 5. Comparisons of thymidine, BV-araU, and these metabolites in HSV-1- and mock-infected cells

Compound	Cell	Virus strain	Amt (pmol/10^6 cells) of the following in cell extracts[a]:				
			N	NMP	NDP	NTP	Total
Thymidine	Vero	Mock	9.5	3	ND[b]	48	60.5
		VR-3	399	2,076	53	160	2,688
		WT-34	33	142	3.9	15.5	194
	HEL	Mock	7.8	11	4.1	ND	23
		VR-3	784	4,079	52	366	5,281
		WT-34	143	392	12	53	600
BV-araU	Vero	Mock	3.6[c]		4.1	ND	7.7
		VR-3	2,598		ND	27	2,625
		WT-34	1,530		16	99	1,645
	HEL	Mock	6.5		ND	0.5	7.0
		VR-3	7,969		ND	332	8,301
		WT-34	6,017		65	453	6,535

[a] N, NMP, NDP, and NTP, Nucleoside, nucleoside monophosphate, nucleoside diphosphate, and nucleoside triphosphate, respectively.
[b] ND, Not detected.
[c] BV-araU monophosphate was not separated from BV-araU.

activities in VR-3-infected HEL cells were almost the same as those in WT-34-infected HEL cells, TK activities in VR-3-infected Vero cells were lower than those in WT-34-infected Vero cells (Fig. 1). From these results and the results of the plaque reduction assay in the presence of HAT, it might be assumed that TS is more important for the ID_{50} in VR-3-infected cells than for that in WT-34-infected cells. However, these mechanisms do not provide an explanation for the differences between results for strains VR-3 and WT-34. Therefore, uptake and turnover of [*methyl*-^{3}H]thymidine and [*bromovinyl*-^{14}C]BV-araU into acid-soluble and acid-insoluble fractions were investigated (Tables 4 and 5). The results of thymidine uptake experiments indicated that larger thymidine pools, thymidine phosphate pools, or both are required for the replication of VR-3 than for that of WT-34. Uptake of BV-araU into VR-3-infected cells was almost the same as that into WT-34-infected cells. These findings support a model in which in the VR-3-infected Vero cells, the BV-araU and BV-araU phosphate pools are diluted by thymidine and thymidine phosphates, thereby causing low antiviral activity against VR-3 in Vero cells, which is in agreement with its antiviral index (15).

In the present study, DNA polymerase (EC 2.7.7.7) was not investigated. Early reports (1, 21) have indicated that HSV-1-induced DNA polymerase is essential for virus DNA replication. Our results indicate that VR-3 and WT-34 replicate equally well in Vero and HEL cells. Furthermore, since both strains of HSV-1 were susceptible to the drugs, at least in HEL cells (Table 2), the DNA polymerases induced by these strains may have the same affinity for the antiherpesvirus compounds. Critical analysis of this point remains to be settled.

The antiherpesvirus activity of araT was greatly augmented in the presence of HAT for WT-34 in Vero cells (Table 3). By the addition of HAT in the medium, the ID_{50} for the WT-34 strain in Vero cells decreased from 1.6 μM (Table 2) to 0.0099 μM (Table 3). The reason for this is not known at present.

Our data on plaque reduction in HAT medium reveal the important role of TS in VR-3-infected Vero cells, even though the TS activity was equal for the extract of the VR-3- and WT-34-infected cells (Fig. 3). These results indicate that TS is regulated by some factor(s) in the cells.

In the many reports about resistant mutants to antiherpesvirus drugs, almost all had mutations for the induction of or for the affinity to TK or DNA polymerase (5, 6, 10, 17, 25). In this study, we have revealed that in addition to the difference in viral TK activities, the size of the thymidine and thymidine phosphate pools and TS activity in HSV-infected cells are also important to the susceptibility of HSV to antiherpesvirus drugs.

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